2024

6TH

SKIN MICROBIOME & Cosmeceuticals CONGRESS

EUROPE

Presented by

MICRO BIOMA .IT

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Table of contents

Key points of the congress

The 6th European Congress on Skin Microbiome & Cosmetic, organized by Global Engage, was held on 23 and 24 April at the Postillion Hotel in The Hague.
Researchers, biologists, doctors from all over the world, interested in the microbiota, met and showed and discussed the most important innovations on the subject.

The most discussed areas were:

- **Skin microbiome testing strategies**
- **Pathogenetic mechanisms**
- **Acne**
- **Atopic dermatitis**
- **Microbiome Friendly Cosmetics**
- **Antiaging**

Participating in the panel discussion at the conclusion of Tuesday afternoon's work were **GEOFF BRIGGS** (Technology Scout – Beauty Incubator, No7 Beauty Company, Member of Walgreens Boots Alliance), **PETER LERSCH** (Vice President Growth Fields, Evonik), **DAN BROWNELL** (Senior Director of Research and Development, AOBiome Therapeutics) and **MARIE DRAGO** (Chief Creative Officer, Galliné).

The theme was "Updates and future directions of Skin Microbiome research • Developing the next generation of microbiome skincare".

To the key question "why do we need to consider the skin microbiota?" **DAN BROWNELL** replied without leaving any possibility of reply "Because it's the skin". This is the spirit in which the entire conference was intended: we have discovered a fundamental component of our skin and we can no longer pretend that it isn't there!

The second most important message was that we still don't know well what is healthy (balanced) but we know quite well what is sick (unbalanced).

What's new in Skin Microbiome Testing?

During the congress there was a lot of talk about **methods for measuring the microbiome**, and in particular the skin microbiome.

Current skin microbiome profiling methods have some important limitations. In particular, the sequencing of amplicons (16S) has the limit of resolution at the genus level and not at the species or strain level, while the metagenomic sequencing with the shotgun technique, which overcomes this limit, has to include the host's DNA (up to 90%) and being very expensive. Furthermore, both then require very long processing times, on the order of weeks.

ANA FINZEL (Senior Scientific Manager of Bio-Me), presented a new system called **Precision Microbiome Profiling** (PMP™) which aims to overcome the current limitations of skin microbiota analysis. Their platform is based on a qPCR method which allows the identification, on an OpenArray panel, of 50 or more taxa of interest at species and strain level, selected from the most represented and most important known ones, including fungi and mites, without being influenced by the host's DNA and providing results within 2-5 days. Among the other advantages of the method would be the **simplicity of the output data** which does not require any bioinformatic analysis with a consequent reduction in the cost of the test.

Subsequently **DAVID GANGITANO** (Chief Scientific Officer, First and Last Project SL. Eres Piel), presented some data relating to the differences measured with this test between the skin microbiota of patients of different age classes, of areas with different quantities of sebum, and demonstrated how the subsequent development of algorithms based on machine learning then made it possible to make predictions, based on the test, with remarkable accuracy.

OLIVER WORSLEY (CEO & Co-Founder, Sequential), also returned to the topic of testing in his presentation entitled "The Future of Skin Microbiome Testing: Insights, Advancements, Approaches". His company has developed a method for collecting skin microbiota samples with a skin patch that promises to be much superior to the swab technique.

They then developed a **Smart ProbeTM**, a panel of 20 microorganisms selected for their impact on health and identified at species, sub-species and strain level.

He then showed that **the vulvar microbiota is very different from the vaginal one**, and much more similar to the skin one, and that a shift from CST IV to CST I favors a more protective ecosystem.

Also **MICHELLE DARGATZ** (Scientist Research, Development & Innovation, Division Nutrition & Care, Evonik Operations GmbH) in her talk titled "Towards skin microbiome friendliness: Improved testing method of cosmetic products with innovative microbiome community models" underlined the **limits of current methods** when one wants to study the complex interactions that occur in microbial systems, it is in fact impossible to study them in vitro and in vivo studies are too expensive.

Their group proposes, as a solution to this problem, another system based on PCR starting from the selection, through sequencing studies, of the most frequent and important genera in the different niches of the skin microbiota (dry, moist, oily and plantar).

They thus achieved a diverse, stable and reproducible community, in a PCR-based system, with which they began testing several possible new cosmetic components.

Their test involves **measuring the effect of the compounds on the total growth of the bacterial community**, measured through monitoring oxygen consumption, pH, turbidity and optical density of the final biomass, and on microbial diversity measured through DNA extraction and sequencing with 16S rRNA technique.

The future prospect of their research involves incorporating host tissue into microbial models to further explore the skin interactome.

Also study the virome

The talk of BJÖRN ANDERSSON (Professor, Department of Cell and Molecular Biology Karolinska Institutet), entitled "The skin virus microbiome and disease; results from the MAARS cohort" was one of the few reports in which the virome was also discussed. In the introductory part he explained some **peculiarities of viruses compared to bacteria**, such as the ability to have inter-kingdom interactions passing from animals to humans and to be able to modify the genetics of the host. He explained that viruses do not constitute a community, that they behave very differently from each other, that many are transient but that some can become chronic. Some of these characteristics make studying the virome more complicated than the microbiome.

Studying the virome involves organizing and filtering data to:

- remove the significant amount of human and environmental contaminants
- remove phages and viruses with very low abundance.

It is therefore necessary to focus on known human viruses, ideally collected at different times, use an RT-step for RNA viruses and subsequently validate the data with PCR. At the end of this data filtering work, some classes of viruses remain which include:

- **Papillomavirus**
- **Herpesviruses**
- **Poxvirus (Molluscum contagiosum)**
- **Picornavirus (enterovirus)**
- **Polyomavirus**
- **Other rare and/or unknown viruse**

These analyzes were included in the works published over the years as part of the **MAARS** (Microbes in Allergy and Autoimmune Related to the Skin) project, some summaries of which were presented. In particular, it seems that the expression of some viruses is increased in psoriasis while that of others is reduced in AD.

What's new in pathogenetic mechanisms?

AHMAD KHODR (Head of the Microbiology Innovation Lab, L'Oréal R&I), in his report "Pysicochemical and molecular characterization of the adhesion mechanisms of cutaneous bacteria." focused on the **mechanisms of adhesion of bacteria to the skin surface**. Modulation of these mechanisms could represent a new strategy to inhibit bacterial adhesion and pathogenicity. Episkin has developed a 3D model called **D-13 model** that allows studying the adhesion of bacteria avoiding animal experiments. **The affinity depends largely on hydrophilicity and hydrophobia**: *S. aureus* is the most hydrophobic and this would allow it to adhere better to the skin.

Another important parameter is the **pH and the ability to donate/receive electrons**. Different topical formulations were then tested for their ability to modulate these surface physicochemical properties of the bacteria. In particular, for example, Rhamnolipids, glycopeptides produced by P. aeruginosa, have been shown to have an anti-adhesion effect on S. aureus. However, it seems that the modulation of the genes involved is minimal. This approach opens **new perspectives in overcoming antibiotic therapies.**

BENJAMIN AL (Global Innovation Manager Life Sciences of Beiersdorf AG), in his report "Skin Microbiome in Health and Disease" also spoke about the skin **characteristics that influence the growth of *S. epidermidis* and *C. acnes***, with particular reference to hydration and sebum content .

For example, *C. acnes* is found primarily in areas of oily skin such as the face and back, while staphylococci are more present in moist areas such as the folds of the limbs. He then underlined that the influences exerted are specific depending on the phylotype (strain) and not common to the same species.

The mutual influence between bacteria

In the field of pathogenetic mechanisms, one of the recurring themes has been the importance of studying bacteria in complex systems as their mutual influences are fundamental in vivo.

Always **BENJAMIN AL**, for example, showed that co-culture experiments with hundreds of different staphylococcal strains allowed to identify **30 strains that exhibited anti-*C. acnes* activity**. Notably, there are staphylococcal strains that selectively exclude acne-associated *C. acnes* strains (e.g., IA1) while coexisting with phylotypes associated with healthy skin (e.g., K, phylotype II).

This regulation would occur through the modulation of antimicrobial activity. Then there are strains of staphylococcus, such as *S. capitis* which appear to inhibit the K strain (phylotype II) of *C. acnes*, which could favor the prevalence of acne-promoting strains. Similarly, some specific strains of *C. acnes* are able to inhibit the antibacterial activity of *S. epidermidis*.

S. epidermidis can reduce colonization by *S. aureus* through the production of AIPs (auto-inducing peptides) which block quorum sensing and 6-HAP (6-N-hydroxyaminopurine) which inhibits purine synthesis.
A particular strain of *S. epidermidis* HAF242 was shown to be able to inhibit the growth of a strain of *C. acnes* DSM1897 (class A) without interfering with *C. acnes* 30.2.L1 (class D) but at the same time, the different influence of co-culture with these two strains on the regulation of *S. epidermidis* genes (epiA, psmβ1-3 and agrD) involved in quorum sensing of *S. aureus* was also demonstrated.

MICHELLE DARGATZ (Scientist Research, Development & Innovation, Division Nutrition & Care, Evonik Operations GmbH) in her talk titled "Towards skin microbiome friendliness: Improved testing method of cosmetic products with innovative microbiome community models" also spoke about the complex **relationships between microbes within the skin microbiota** outlining these main 4 modes of interactions:

1 **Competition**... for space and nutrients

2 **Synergism**, which consists of encouraging the growth of another member, for example through cross-feeding mechanisms

3 **Antagonism**, through the production of inhibitory substances such as bacteriocins and short-chain fatty acids (SCFAs)

4 **Interruption of cross-talk due to quorum sensing interference**

The aim of their research is to find a system to study these complex interactions as it is not possible to study them *in vitro* but *in vivo* studies are too expensive.

KIM HARDIE (Professor in Bacterial Pathogenesis, University of Nottingham & Co-Investigator, National Biofilms Innovation Center) in her report entitled "Commensal bacteria protect skin cells from the pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*" introduced the concept of the possible cooperative or competitive action of *S. aureus* in polymicrobial infections, particularly those characterized by biofilms where bacteria coexist.

What's new in Acne?

One of the most studied pathologies at the skin microbiota level is undoubtedly acne. In studies on this pathology, understanding the mutual **influence between bacteria and the influence of the physico-chemical characteristics of the skin on their proliferation** has proven to be extremely important.

BENJAMIN AL (Global Innovation Manager Life Sciences of Beiersdorf AG), in his report "Skin Microbiome in Health and Disease" spoke about the mutual influence between commensal bacteria in the pathogenesis of acne (see *"The mutual influence between bacteria"* section).

SIMONA BEFI (Head of R&D, S-Biomedic), spoke about acne in her presentation entitled "Cutibacterium acnes, pioneering probiotics potential for skin health" and showed how the evolution of the treatment has gradually evolved from killing all bacteria including beneficial ones, to an attempt to apply bacteria, live or dead, without considering that they are not the ones needed by the skin, to finally a precision approach that uses vital strains belonging to the skin microbiota with specific beneficial functions.

HOLGER BRÜGGEMANN (Associate Professor, Department of Biomedicine, Aarhus University, Denmark), in his talk entitled "Skin microbiome interferences and the potential exploitation for acne treatment", spoke about skin dysbiosis, the effect of isotretinoin and the strong impact of Staphylococci in the pathogenesis of the disease.

In a study on 35 acne patients, who had not undergone antibiotic treatments in the last 6 months, subjected to a skin swab they measured the quantity and relative abundance of the populations of *C. acnes* and Staphylococci, highlighting a slight reduction of the former and a significant relative increase of the seconds. With the SLTS (single-locus sequence typing) technique it is possible to distinguish 10 classes of *C. acnes* which correspond to the phylotypes, classes A-E to the IA1 phylotype, F to the IA2 phylotype, H to the IB

phylotype, G to the IC phylotype, K to phylotype II (*C. acnes susp. defendans*) and L to phylotype III (*C. acnes subsp. elongatum*).

Therefore, reanalyzing the data at phylotype level, it emerged that at the *C. acnes* level there was a significant reduction in classes H, K and L (phylotypes II and III) and a relative but not significant increase in classes A, C, E and F (phylotype I).

The reduction of *C. acnes* is more evident on the cheek while the reduced alpha-diversity of *C. acnes* is greater on the skin of the back.

Analyzing the seven main species of Staphylococci, however, there is a significant reduction in *S. hominis* and *S. warneri* compared to a relative but not significant increase in *S. epidermidis* and *S. aureus*. Staphylococci in general are not reduced in number while for them too the reduction in alpha-diversity is greater at the level of the skin of the back.

What changes significantly is the ratio between *C. acnes* and Staphylococci which goes from 34:1 in healthy subjects to 11:1 in subjects with acne.

Isotretinoin modulates this dysbiosis by causing a slight reduction in Staphylococci but dramatically increasing their diversity through a significant relative reduction in *S. epidermidis* and a significant relative increase in *S. aureus*, but at the same time drastically depletes *C. acnes* by further reducing the ratio *C. acnes* / Staphylococci from 11:1 to 1:1. Isotretinoin therefore modulates the microbiota by reducing *C. acnes* but also damages *S. epidermidis* and promotes the growth of *S. aureus* which could be dangerous. Post-isotretinoin strategies must therefore be considered to restore the skin microbiome.

The possibility of selecting and applying beneficial strains of *S. epidermidis* to the skin, in particular those capable of reducing colonization by *S. aureus*, was therefore examined. Through genomic analysis, *S. epidermidis* strains associated with those not associated with pathology were distinguished. Subsequently, those isolated from healthy skin were tested for their antimicrobial activity against *C. acnes* and *S. aureus*, demonstrating that approximately 5% of the strains possess specific activity. Some of these then have a selective inhibition activity on some strains (pathogenic) of *C. acnes* without interfering with the others (beneficial).

Finally, the bacteriocins produced by these strains were studied, discovering that only three of these produce already known bacteriocins while all produce still unknown bacteriocins.
A strain of *S. epidermidis* HAF242 was therefore isolated capable of inhibiting the growth of a strain of *C. acnes* DSM1897 (class A) without interfering with *C. acnes* 30.2.L1 (class D) and the different influence of co-culture with these two strains on the regulation of *S. epidermidis* genes involved in quorum sensing was also demonstrated.
The hypothesis is therefore that class D *C. acnes* are able to inhibit the AgrQs system of *S. epidermidis* and its anti-*C. acnes* activity, reaching a situation of "tolerance" that class A does not achieve.

The last step of the study demonstrated that after the topical application of specific strains of *S. epidermidis* (SE1) with anti-*S. aureus* and anti-class A *C. acnes* activity, persistence (but only of the DNA, which does not demonstrate viability) after 7 and 30 days was still high and the *S. aureus* load decreased. However, a poor impact on *C. acnes* was demonstrated, perhaps due to the different skin niche in which the two bacteria proliferate more.

DAN BROWNELL (Senior Director of Research and Development, AOBiome Therapeutics) in the report entitled "B244 Utilizes Multiple Mechanisms of Action to Treat Atopic Dermatitis, a Multifactorial Disease" focused on the characteristics of this single strain of ammonia-oxidizing bacteria spoke not only of AD but also of acne, illustrating **in vitro and in vivo studies which have demonstrated that AOBs are very effective in reducing the proliferation of *C. acnes***, probably also through a strong reduction in sebum production, and phase 2b clinical studies which have demonstrated a **significant reduction in the IGA score and inflammatory lesion count at 12 weeks**.

This significance remains high not only compared to pre-treatment or placebo but also in trials comparing it to some reference products (such as adapalene) compared to which AOBs always show better tolerability and the absence of side effects.

B244 could therefore respond to some unmeet needs of current therapies which often cause dryness or burning, which cannot be used for long periods or which also have systemic side effects.

A **SynBalance poster** showed the benefits of an oral probiotic containing *Lactiplantibacillus plantarum* PBS067, *Limosilactobacillus reuteri* PBS072 and *Lacticaseibacillus rhamnosus* LRH020 administered for 8 weeks to patients aged 18 to 50 with mild-to-moderate acne. The study demonstrated improvement in skin hydration, sebum production and pH values consistent with the reduction of non-inflammatory lesions in the treated group compared to the placebo. Even greater improvement at T70, i.e. 2 weeks after stopping treatment.

A **SynFlora poster** showed data on engineering *C. acnes* to produce and secrete in vivo a therapeutic molecule, Neutrophil Gelatinase-Associated Lipocalin (NGAL), capable of modulating sebum production.

What's new in Atopic Dermatitis?

One of the pathologies most talked about during the congress was atopic dermatitis, of which it was underlined that, especially during flare-ups, a general loss of microbial diversity is observed characterized by an increase in *S. Aureus* and *S. epidermidis* and a reduction of *S. hominis*.
Particular attention was paid to the role of *S. aureus* which increasingly proves to be the driver of inflammation in AD through numerous mechanisms including the production of toxins and proteases.

BENJAMIN AL (Global Innovation Manager Life Sciences of Beiersdorf AG), in his talk "Skin Microbiome in Health and Disease" talked about the relationship between different commensal bacteria in AD.
Even in AD, as already demonstrated in acne, it seems increasingly clear that it is necessary to study the activity of the bacteria and their mutual influences at the strain level and not just at the species level.
The prevalence of specific strains of *S. aureus* appears to be associated with eczema flare-ups.

This specificity could be linked to the presence and role of particular enterotoxins (A and B) and staphylococcal proteases as important virulence factors.

CATHERINE O'NEIL (Professor of Translational Dermatology University of Manchester), in her talk entitled "Mining the skin microbiome for new approaches to the management of Atopic dermatitis" underlined how the elimination of *S. aureus* is one of the most important goals of the treatment of AD but also how traditional antibiotic therapy it is starting to show many limitations.

In particular, the re-infection rates are very high, reaching 40% of patients after 3 months and 50% after 6. **One of the causes of resistance to antibiotics by *S. aureus* is its ability to penetrate inside human keratinocytes** (without damaging them) **where only rifampicin is able to penetrate and carry out its action.**
We are faced with an important unmet need in the treatment of this pathology!

By studying the mechanisms by which *S. aureus* activates inflammation, it emerged that it is the only type of Staphylococcus capable of inducing a rapid release of constitutive IL-33 from human keratinocytes independently of the Toll-like receptor pathway, thus activating a response Th2 in the absence of infection. This property derives above all from the production and release of the Sbi protein (second immunoglobulin-binding protein) and the consequent reduction in the production of corneodesmosin, a component of the corneodesmosomes which form the main intercellular adhesive structures in the stratum corneum, with a consequent reduction in the skin barrier function.

By studying skin swabs and their secretome, **CATHERINE O'NEIL**'s group managed to isolate a protein secreted by *Micrococcus Luteus* which is able to cleave and inactivate the Sbi protein of *S. aureus*. The protein, called AE1, is a highly variable protein in nature, subject to numerous mutations especially in the *Micrococcus luteus* strain NCTC 2665 (the so-called "Fleming" strain). They then studied a recombinant AE1 protein and demonstrated that it is able to inhibit the secretion of IL33.

These studies open new interesting perspectives regarding the role of *Micrococcus luteus* in AD and the possible use of the AE1 protein in its treatment.

In his speech entitled "The Future of Skin Microbiome Testing: Insights, Advancements, Approaches" **OLIVER WORSLEY** (CEO & Co-Founder, Sequential), showed how chronic hand eczema (CHE) has a gut microbiota characterized by the relative abundance of 24 different bacterial species compared to healthy subjects, with a lower abundance of Bifidobacteria, thus hypothesizing a role of intestinal dysbiosis in CHE.

ROGER FRECHETTE (Chief Business Officer, Concerto Biosciences) in the talk "Developing therapeutic and cosmetic microbe-based products for eczema" presented their **kChip**-based microbiota analysis technology.
The technology involves crossing 64 or more different bacteria on a plate to randomly recreate more than 2000 (up to 12M!) co-cultures and discover their mutual relationships.

Thanks to this technology they created the first **map of the skin interactome** capable of controlling the virulence of *S. aureus* and discovered a **specific combination of three bacterial strains intended**

to correct the lack of microbial diversity and reduce the virulence of *S. aureus* which characterizes the skin affected by eczema.

The bacterial combinations are called "Ensemble" followed by a number, and this specific combination is the Ensemble number 2 (ENS-002) while the most recent ENS-003 is a candidate to combat recurrent vaginal candidiasis. Ensemble-002 was then tested by adding it to an in vitro skin tissue model and comparing the pathogenicity of *S. aureus* alone, with ENS-002 and with mupirocin, a common topical antibiotic.

ENS-002 was able to suppress the pathogenicity of *S. aureus* and, unlike the antibiotic, does not induce the emergence of hyper-virulent or hyper-pathogenic cells.

For this product, the optimization phase is being completed and a first Phase 1 study on humans is starting. In addition to the discovery of Ensemble-002, the analysis of the skin microbiota also made it possible to isolate a bioproduct (called **ii283-N**) which is particularly **effective in limiting the growth and metabolism of *S.aureus* and extinguishing its virulence** and has led to the hypothesis of being able to insert the ii238-N supernatant (in this case a post-biotic) into topical products to be used on eczema flare-ups and superinfections.

DAN BROWNELL (Senior Director of Research and Development, AOBiome Therapeutics) gave a talk titled "B244 Utilizes Multiple Mechanisms of Action to Treat Atopic Dermatitis, a Multifactorial Disease" focused on the characteristics of this single strain of ammonia-oxidizing bacteria. AOBs (ammonia oxidizing bacteria) are already used in sprays for topical use with a wide area of therapeutic applications, with a high safety profile characterized by over 1000 patients treated with 0 side effects and Phase 3 studies on AD and phase 2 on acne and rosacea.

Through the oxidation of ammonia, produced 70% in the liver and 30% in the skin, **B244 generates various metabolites, with an immunomodulatory and beneficial action for the skin, including in particular nitric oxide, with vasodilatory and anti-inflammatory activities and nitrites with anti-infectious activity**.

B244 was the subject of a Phase 2b trial in adults with mild to moderate AD and moderate to severe pruritus. The spray was well tolerated and demonstrated improved efficacy

over vehicle across all primary, secondary and exploratory endpoints and will be further developed as a novel, natural, fast-acting topical spray treatment for AD and associated pruritus. Interesting to note the effect on itching which remained significant even 4 weeks after the end of treatment.

KIM HARDIE (Professor in Bacterial Pathogenesis, University of Nottingham & Co-Investigator, National Biofilms Innovation Center), in her speech entitled "Commensal bacteria protect skin cells from the pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*" talked above all about wound healing, but also showed how mutual interference between bacteria can be important in AD. In fact, in *in vivo* studies on animals it has been demonstrated that the presence of Agr produced by *S. aureus* correlates with an inflammation similar to AD and that coagulase negative staphylococci (CoNS) produce a wide range of AIPs (auto-inducing peptides) which block quorum sensing. In particular, **AIP-1 and AIP-2 produced by *S. hominis* have been shown to inhibit group 1 Agr of *S. aureus*.**

In the talk by **BJÖRN ANDERSSON** (Professor, Department of Cell and Molecular Biology Karolinska Institutet), entitled "The skin virus microbiome and disease; results from the MAARS cohort", one of the few speech in which the virome was also discussed, a role in the AD also emerged. In particular, it seems that the expression of some viruses is reduced in AD .

A **Re-Connecting Nature poster** presented data from a study of 140 AD patients who were given a spray containing bacteria extracted from soil in Finland.

What's new in Psoriasis?

One of the few talk in which psoriasis was discussed was that of **BJÖRN ANDERSSON** (Professor, Department of Cell and Molecular Biology Karolinska Institutet) entitled "The skin virus microbiome and disease; results from the MAARS cohort", where some data relating to the virome in this pathology were presented. In particular, it seems that the expression of some viruses is increased in psoriasis.

In a study carried out on biopsies taken on lesional and perilesional skin, it emerged however that all viruses decreased in the lesional areas of psoriasis.

One hypothesis to explain this observation is that the viral infection may start in the center of the lesions but is then removed by the immune response and ends up moving to the perilesional skin.

What's new in wound healing?

There was no talk that specifically dealt with wound healing but in several speeches data emerged regarding the skin injury, repair mechanisms, biofilm and infection control.

In his speech entitled "The Future of Skin Microbiome Testing: Insights, Advancements, Approaches" **OLIVER WORSLEY** (CEO & Co-Founder, Sequential), showed how significant alterations in the intestinal microbiota are observed following skin injury.

DAN BROWNELL (Senior Director of Research and Development,

AOBiome Therapeutics) in the talk entitled "B244 Utilizes Multiple Mechanisms of Action to Treat Atopic Dermatitis, a Multifactorial Disease" focused on the characteristics of this single strain of ammonia-oxidizing bacteria spoke not only of AD but also of wound healing, showing how in controls without AOB an initial inappropriate immune response was observed which is not part of the healing process but hinders it and therefore hypothesizing a possible use of AOB also in the treatment of wounds.

The presentation where wound healing was talked about the most was that of **KIM HARDIE** (Professor in Bacterial Pathogenesis, University of Nottingham & Co-Investigator, National Biofilms Innovation Center), entitled "Commensal bacteria protect skin cells from the pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*" in which he was introduced the concept of the possible cooperative or competitive action of *S. aureus* in polymicrobial infections, particularly those characterized by biofilms where bacteria coexist.

Infections where biofilms constitute a particular problem are especially those of chronic ulcers responsible for very high healthcare costs. Damaged skin is in fact at risk of attack by pathogens while its disinfection hinders the positive effects of beneficial bacteria. In particular, *S. aureus* is a very versatile pathogen due to some of its peculiar characteristics including the presence of various colonization factors of cellular barriers, immunomodulatory agents and the secretion of exoproducts.

The growth of *S. aureus* is controlled by quorum sensing and the Agr peptide is one of the molecules that signals QS. **When Agr is present it leads to an acute infection while its absence is linked to chronic infections characterized by biofilm**. Furthermore, Agr is one of the key molecules of competitive interference between different species of Staphylococci.

Coinfections such as that with *S. aureus* and *P. aeruginosa*, a known biofilm producer, usually have a worse clinical outcome. But if we add one or more commensals to the pathogen these infections also reduce their aggressiveness. Commensal bacteria can protect skin cells from pathogen attacks through competitive interference with *S. aureus* Agr. Coagulase-negative staphylococci (CoNS) produce a wide range of AIPs (auto-inducing peptides) that block quorum sensing.

By studying synthetic models of chronic wounds (SCW), it was discovered that

- different strains of *P. aeruginosa* are distributed differently
- the chronicity-defective strains die predominantly in the center of the microcolonies
- their death is exacerbated by the presence of *S. aureus*.

The cocultures of the two bacteria can therefore have different effects, of coexistence, synergy or competition depending on the defective chronicity.
Matched pairs coexist more easily than unmatched ones.
Furthermore, the presence of multiple species within the biofilm alters the bactericidal activity of antimicrobials and *S. aureus* within multispecies biofilms, containing for example commensals such as *Micrococcus luteus* or *S. epidermidis* but also pathogens such as *P. aeruginosa*, are sensitive to much lower concentrations of antibiotic.

Another very interesting data shown by **KIM HARDIE** in her talk concerns the **effect of biocides on antibiotic tolerance within the biofilm**.
Triclosan, for example, a disinfectant widely used in household and healthcare products, greatly reduces the susceptibility of *S. aureus* to ciprofloxacin and vancomycin.

Triclosan is an inhibitor of fatty acid synthesis and accumulates in the environment and in the human body and traces have been found in urine, blood and nasal secretions. No one has yet verified what effect it may have on models of polymicrobial infections.

What's new in antiaging?

OLIVER WORSLEY (CEO & Co-Founder, Sequential), in his presentation entitled "The Future of Skin Microbiome Testing: Insights, Advancements, Approaches" also talked about specific bacteria, such as *Corynebacterium* which are linked to skin aging.

WORSLEY showed a negative correlation between the improvement of "crows feet" after a treatment and the presence of Roseomonas mucosa, a bacterium considered important in the regulation of the skin barrier function and elasticity.

What's new in cosmetics *"microbiome friendly"*?

The cosmetics topic was one of the most discussed of the entire congress. **Currently 90% of the components of cosmetics can potentially damage bacteria**. To this possible direct damage is added the potential indirect damage linked to the influence that cosmetics can have on the physico-chemical properties (hydrophobia, pH) that modulate the adhesion of bacteria to the skin. Many talks have therefore questioned **what characteristics a cosmetic must have in order to boast the *microbiome-friendly claim*** and how they must be tested.

MICHELLE DARGATZ (Scientist Research, Development & Innovation, Division Nutrition & Care, Evonik

Operations GmbH) in her talk titled "Towards skin microbiome friendliness: Improved testing method of cosmetic products with innovative microbiome community models" spoke about the growing demand from consumers for cosmetics microbiome-friendly which now represent a key area for producers of cosmetic ingredients.

First of all, she underlined the difficulty of a correct definition of a healthy skin microbiome as the interpersonal differences are considerable.

There is a lack of precise guidelines to define the health of the microbiota and the testing protocols differ greatly in complexity and interpretation.

The definition she proposes is that

> **"the chemical and biological components and their mixtures that must be applied to the skin should not have a significant impact on the quantitative or qualitative composition of the skin microbiota"**

Her group developed a microbial community model containing the most prominent species inhabiting the human skin with which they began testing several possible new cosmetic components.
Their test involves **measuring the effect of the compounds on the total growth of the bacterial community**, measured through monitoring oxygen consumption, pH, turbidity and optical density of the final biomass, and on **microbial diversity** measured through DNA extraction and sequencing with 16S rRNA technique.

The data are then expressed on a "Matrix" which considers the increase or reduction in total growth on the abscissae and the changes to the composition on the ordinates, identifying 4 types of substances:

MICROBIOME PROMOTING:
if they increase growth without changing the composition

MICROBIOME FRIENDLY:
if they leave both growth and composition unchanged

MICROBIOME SHIFTING:
if they modify the composition without overly altering the growth

MICROBIOME IMPAIRING:
if they significantly reduce both growth and composition

Finally it showed some examples of evaluation of substances with respectively antimicrobial and bioactive activity and the relative position of the results in the matrix (see figure).

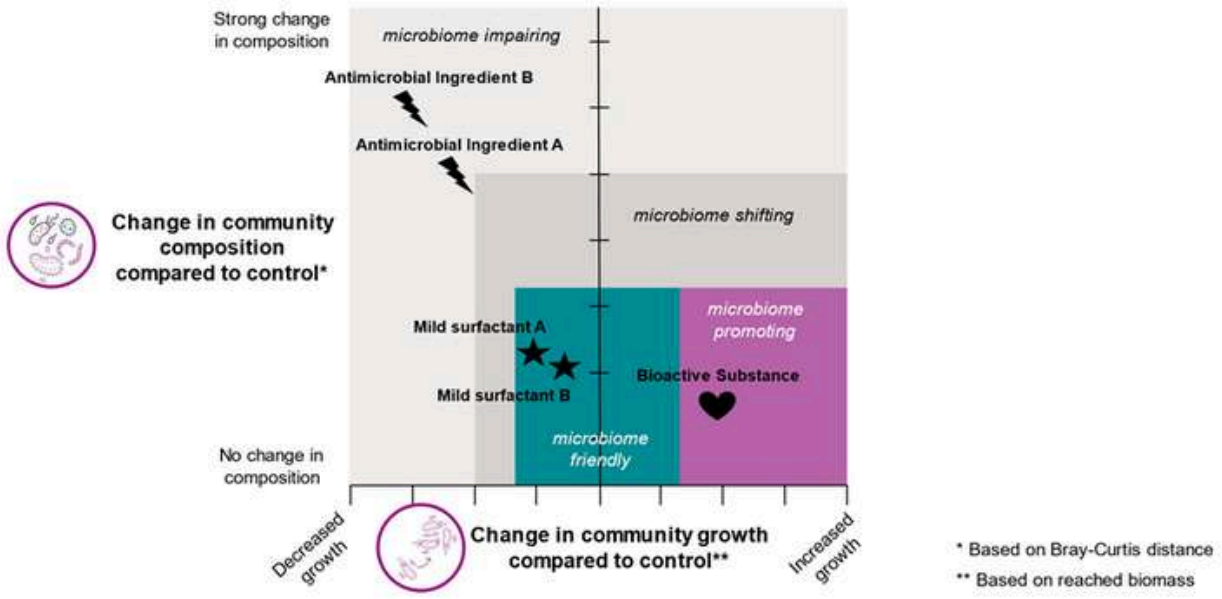


Figure: Evaluation Matrix for Determining Microbiome Friendliness in a Microbial Community © Evonik Operations GmbH

The model presented allows to **assess the impact of cosmetic compounds on the quantitative and qualitative makeup of a microbial community**, including the most prevalent species found on human skin, and thus allows for the evaluation of the compounds' skin microbiome friendliness.

SABRINA BEHNKE's (Business Director, Biopolymers & Modern Preservatives, TRI-K) talk focused precisely on the lack of standardized tests to evaluate the real impact of cosmetics on the microbiota.
In vitro techniques have the advantage of being cheaper and easily replicable and of being able to test in a simple way even microbiota of very sensitive areas such as the genital one.
On the other hand, they are not able to faithfully reproduce the polymicrobial communities nor to consider the capacity of the bacteria to "hide" in the follicles.

Those *in vivo* are more representative of the real polymicrobial community present on the skin but are subject to enormous interpersonal variability, are difficult to replicate and very expensive.

According to **BEHNKE**, tests should include 4 phases:

1. **Microbial quality:** they must not be contaminated with unwanted bacteria
2. **Balance tests** (co-cultures): they must not upset the balance between different species that keep each other under control
3. **Diversity tests** (co-cultures): must not reduce microbial diversity
4. **Direct and indirect viability tests:** to simulate the effect on both the more superficial microbes and those present in the deeper layers of the skin.

Then speaking about the characteristics that cosmetics should have to be declared microbiome-friendly, he addressed the issue of how to reconcile the need to keep cosmetics safe from contamination with that of preserving the health of the skin microbiota.

The literature is ambiguous because while according to some works (e.g. Pinto 2021) most of the preservatives contained in cosmetics have an action on bacteria resident on the skin, others (e.g. Murphy 2021) state the exact opposite, i.e. that despite the antimicrobial effectiveness of preservatives *in vitro*, the skin microbiome is not affected by products containing preservatives *in vivo*.

The factors for which preservatives cannot be considered intrinsically harmful to the microbiota include dilution, contact time and possible rinsing, actual persistence on the skin as well as the ability of microorganisms to "take refuge" in the follicles and sebaceous glands and to repopulate the skin after the insult.

His speech attempted to outline the characteristics that differentiate modern preservatives from traditional ones. While traditional preservatives were low cost, with a broad spectrum of effectiveness, a wide pH range and poor incompatibilities, modern ones will have greater limitations in pH and incompatibility and, also by virtue of the lower effectiveness, will require "smart" combinations which will likely lead to higher costs.

Finally, it presented two new

antimicrobial boosters based on amino acids, free of solvents, based on Capryloyl Glycine and Undecylenoyl Glycine (TRIcareTM CG and LipoG) produced with green chemistry processes and a biodegradable catalyst.
Caprylyol Glycine is a multifunctional ingredient which, at the same time as its antimicrobial booster activity, has been shown to reduce sebum production, redness and pore size, helping to combat the microorganisms responsible for acne and dandruff.
The mechanism of action concerns the inhibition of the 5-alpha reductase pathway.

Also **SPYRIDON MARKOS** (Sales and Key Account Manager, QACS) in his talk "Do Your Skin Microbes Like Your New Skincare Product? Towards a Simple Benchmark Test" spoke about the importance of evaluating the impact of cosmetics on the skin microbiome and presented their test to evaluate it.
He underlined how the **average adult uses 12 cosmetics a day**, which constitute the main source of substances (up to 160 in women and more than 100 in men) to which we are exposed and that **the compounds contained in cosmetics can remain on the skin for weeks from their use despite even daily washing**.

Many cosmetics alter bacterial diversity as well as the dynamics and structure of human and bacterial molecules.

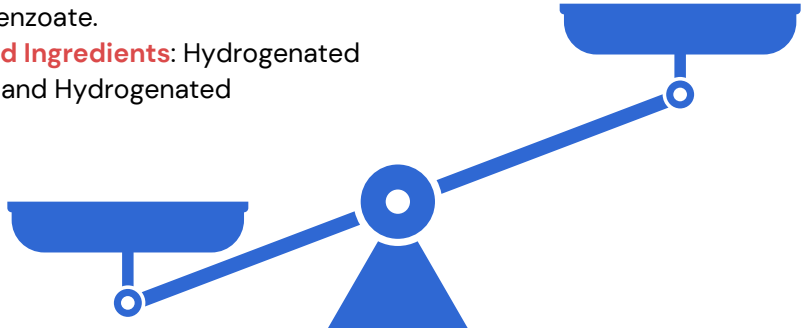
What he proposed is an *in-vitro/ex-vivo* test on the viability of different strains of bacteria, grouped into 19 different "panels" divided by areas (face, scalp, genitals, etc.) and including both normal commensal and main pathogens known for that specific area.
The microbiota of a pool of volunteers (2500 people) was collected through swabs and used to evaluate the friendliness of skincare products. **The test can be used to obtain the microbiome-friendly claim and to guide the possible reformulation of products that are not**.
He also presented a preliminary list, derived from their testing, of ingredients that are friends or enemies of the microbiome (see figure).

In her speech **MARIE DRAGO** (Chief Creative Officer, Gallinée), spoke of a real revolution in hygiene products which involves a **transition from clean to wellness** in a holistic vision that exploits knowledge of the microbiota and on the gut-brain-skin axis, to bring together the goals of better skin and a more peaceful mind.

In fact, stress, states **DRAGO**, is a problem affecting the entire organism which then manifests itself as leaky gut at the intestinal level and hypersensitivity at the skin level. She then showed how users of her products report not only an improvement in skin but also in positive sensations and stress levels after their use! And she wondered whether this effect is just a consequence of seeing one's skin improve or whether it could depend on the gut-brain-skin axis.

Surfactants: Cationic, Sodium Laureth Sulfate (anionic), Lauryl Glucoside, and Coco-Betaine (milder).
Synthetic Fragrances: "Fragrance" or "Parfum".
Alcohol: Alcohol and its derivatives like Ethylhexylglycerin and Alcohol Denat.
Preservatives: Phenoxyethanol, o-Cymen-5-ol, (2-methyl-5-isopropylphenol), Potassium Sorbate, Sodium Benzoate.
Petroleum-Derived Ingredients: Hydrogenated Poly(C6-14 Olefin) and Hydrogenated Polydecene.

Natural Plant Extracts and Oils: Houttuynia Cordata Extract, Centella Asiatica Extract, Rosehip Rosa Canina Fruit Oil, Argania Spinosa Kernel Oil (Argan Oil), Citrullus Lanatus Seed Oil (Kalahari Melon Seed Oil), and Avena Sativa Kernel Oil (Oat Kernel Oil).
Humectants and Hydrating Agents: Glycerin, Sodium Hyaluronate, Betaine, and Panthenol.
Skin Barrier Support: Ceramide NP and Hydrogenated Lecithin.
Natural Preservatives and Ferments: 1,2-Hexanediol, Ethylhexylglycerin, and Tocopherol (Vitamin E) - Lactobacillus Ferment Lysate, Saccharomyces/Rice Ferment Filtrate Extract, and Hydrolyzed Lactobacillus Ferment Filtrate



Addendum

S. aureus: what did they say about me?

S. aureus drives inflammation in AD and several other pathologies including itching, abscesses and pimples, some folliculitis, SSSS (staphylococcal scaled skin syndrome) and impetigo. The mechanisms through which *S. aureus* creates or aggravates these pathologies mainly concern the barrier damage caused by some proteases it produces and the activation of the immune system caused by its toxins.

Numerous speakers during the congress spoke about S. aureus, its pathogenetic mechanisms and possible strategies to control it.

AHMAD KHODR (Head of the Microbiology Innovation Lab, L'Oréal R&I), in his report "Pysicochemical and molecular characterization of the adhesion mechanisms of cutaneous bacteria." focused on the mechanisms of adhesion of bacteria to the skin surface. Modulation of these mechanisms could represent a new strategy to inhibit bacterial adhesion and pathogenicity. Episkin has developed a 3D model called D-13 model that allows studying the adhesion of bacteria avoiding animal experiments. The affinity depends largely on hydrophilicity and hydrophobia: *S. aureus* is the most hydrophobic and this would allow it to adhere better to the skin. Another important parameter is the pH and the ability to donate/receive electrons. Different topical formulations were then tested for their ability to modulate these surface physicochemical properties of the bacteria. In particular, for example, Rhamnolipid, a glycopeptide produced by *P. aeruginosa*, has been shown to have an anti-adhesion effect on *S. aureus*. However, it seems that the modulation of the genes involved is minimal. This approach opens new perspectives in overcoming antibiotic therapies.

BENJAMIN AL (Global Innovation Manager Life Sciences of Beiersdorf AG), in his talk "Skin Microbiome in Health and Disease" talked about the relationship between different commensal bacteria in AD. Even in AD, as already demonstrated in acne, it seems increasingly clear that it is necessary to study the activity of the bacteria and their mutual influences at the strain level and not just at the species level. T
he prevalence of specific strains of *S. aureus* appears to be associated with eczema flare-ups. This specificity could be linked to the presence and role of particular enterotoxins (A and B) and staphylococcal proteases as important virulence factors. Then he showed data on co-culture experiments with hundreds of different staphylococcal strains.
S. epidermidis can reduce colonization by *S. aureus* through the production of AIPs (auto-inducing peptides) which block quorum sensing and 6-HAP (6-N-hydroxyaminopurine) which inhibits purine synthesis. A particular strain of *S. epidermidis* HAF242 was shown to be able to inhibit the growth of a strain of *C. acnes* DSM1897 (class A) without interfering with *C. acnes* 30.2.L1 (class D) but at the same time, the different influence of co-culture with these two strains on the regulation of *S. epidermidis* genes (epiA, psmβ1-3 and agrD) involved in quorum sensing of *S. aureus* was also demonstrated.

KIM HARDIE (Professor in Bacterial Pathogenesis, University of Nottingham & Co-Investigator, National Biofilms Innovation Center) in her report entitled "Commensal bacteria protect skin cells from the pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*" introduced the concept of the possible cooperative or competitive action of *S. aureus* in polymicrobial infections, particularly those characterized by biofilms where bacteria coexist.

HOLGER BRÜGGEMANN (Associate Professor, Department of Biomedicine, Aarhus University, Denmark), in his talk entitled "Skin microbiome interferences and the potential exploitation for acne treatment", spoke about skin dysbiosis, the effect of isotretinoin and the strong impact of Staphylococci in the pathogenesis of the disease.

In a study on 35 acne patients, who had not undergone antibiotic treatments in the last 6 months, subjected to a skin swab they measured the quantity and relative abundance of the populations of *C. acnes* and Staphylococci, highlighting a slight reduction of the former and a significant relative increase of the seconds. Analyzing the seven main species of Staphylococci, however,

there is a significant reduction in *S. hominis* and *S. warneri* compared to a relative but not significant increase in *S. epidermidis* and *S. aureus*. Staphylococci in general are not reduced in number while for them too the reduction in alpha-diversity is greater at the level of the skin of the back.

Isotretinoin modulates this dysbiosis by causing a slight reduction in Staphylococci but dramatically increasing their diversity through a significant relative reduction in *S. epidermidis* and a significant relative increase in *S. aureus*- Isotretinoin therefore modulates the microbiota by reducing *C. acnes* but also damages *S. epidermidis* and promotes the growth of *S. aureus* which could be dangerous. Post-isotretinoin strategies must therefore be considered to restore the skin microbiome.
The possibility of selecting and applying beneficial strains of *S. epidermidis* to the skin, in particular those capable of reducing colonization by *S. aureus*, was therefore examined. Through genomic analysis, *S. epidermidis* strains associated with those not associated with pathology were distinguished. Subsequently, those isolated from healthy skin were tested for their antimicrobial activity against *C. acnes* and *S. aureus*, demonstrating that approximately 5% of the strains possess specific activity.
After the topical application of specific strains of *S. epidermidis* (SE1) with anti-*S. aureus* and anti-class A *C. acnes* activity, persistence (but only of the DNA, which does not demonstrate viability) after 7 and 30 days was still high and the *S. aureus* load decreased. However, a poor impact on *C. acnes* was demonstrated, perhaps due to the different skin niche in which the two bacteria proliferate more.

CATHERINE O'NEIL (Professor of Translational Dermatology University of Manchester), in her talk entitled "Mining the skin microbiome for new approaches to the management of Atopic dermatitis" underlined how the elimination of *S. aureus* is one of the most important goals of the treatment of AD but also how traditional antibiotic therapy it is starting to show many limitations. In particular, the re-infection rates are very high, reaching 40% of patients after 3 months and 50% after 6. One of the causes of resistance to antibiotics by *S. aureus* is its ability to penetrate inside human keratinocytes (without damaging them) where only rifampicin is able to penetrate and carry out its action. We are faced with an important unmet need in the treatment of this pathology! By studying the mechanisms by which *S. aureus* activates inflammation, it emerged that it is the only type of Staphylococcus capable of

inducing a rapid release of constitutive IL-33 from human keratinocytes independently of the Toll-like receptor pathway, thus activating a response Th2 in the absence of infection.
This property derives above all from the production and release of the Sbi protein (second immunoglobulin-binding protein) and the consequent reduction in the production of corneodesmosin, a component of the corneodesmosomes which form the main intercellular adhesive structures in the stratum corneum, with a consequent reduction in the skin barrier function.

By studying skin swabs and their secretome, CATHERINE O'NEIL's group managed to isolate a protein secreted by *Micrococcus Luteus* which is able to cleave and inactivate the Sbi protein of *S. aureus*. The protein, called AE1, is a highly variable protein in nature, subject to numerous mutations especially in the *Micrococcus luteus* strain NCTC 2665 (the so-called "Fleming" strain). They then studied a recombinant AE1 protein and demonstrated that it is able to inhibit the secretion of IL33. These studies open new interesting perspectives regarding the role of *Micrococcus luteus* in AD and the possible use of the AE1 protein in its treatment.

ROGER FRECHETTE (Chief Business Officer, Concerto Biosciences) in the talk "Developing therapeutic and cosmetic microbe-based products for eczema" presented their kChip-based microbiota analysis technology. The technology involves crossing 64 or more different bacteria on a plate to randomly recreate more than 2000 (up to 12M!) co-cultures and discover their mutual relationships. Thanks to this technology they created the first map of the skin interactome capable of controlling the virulence of *S. aureus* and discovered a specific combination of three bacterial strains intended to correct the lack of microbial diversity and reduce the virulence of *S. aureus* which characterizes the skin affected by eczema.
The bacterial combinations are called "Ensemble" followed by a number, and this specific combination is the Ensemble number 2 (ENS-002) while the most recent ENS-003 is a candidate to combat recurrent vaginal candidiasis.
Ensemble-002 was then tested by adding it to an in vitro skin tissue model and comparing the pathogenicity of *S. aureus* alone, with ENS-002 and with mupirocin, a common topical antibiotic. ENS-002 was able to suppress the pathogenicity of *S. aureus* and, unlike the antibiotic, does not induce the emergence of hyper-virulent or hyper-pathogenic cells. For this product, the optimization phase is being completed and a first Phase 1 study on humans is

starting. In addition to the discovery of Ensemble-002, the analysis of the skin microbiota also made it possible to isolate a bioproduct (called ii283-N) which is particularly effective in limiting the growth and metabolism of *S.aureus* and extinguishing its virulence and has led to the hypothesis of being able to insert the ii238-N supernatant (in this case a post-biotic) into topical products to be used on eczema flare-ups and superinfections.

KIM HARDIE (Professor in Bacterial Pathogenesis, University of Nottingham & Co-Investigator, National Biofilms Innovation Center), in their speech entitled "Commensal bacteria protect skin cells from the pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*" introduces the concept of the possible cooperative or competitive action of *S. aureus* in polymicrobial infections, particularly those characterized by biofilms where bacteria coexist.

Infections where biofilms constitute a particular problem are especially those of chronic ulcers responsible for very high healthcare costs. Damaged skin is in fact at risk of attack by pathogens while its disinfection hinders the positive effects of beneficial bacteria. In particular, *S. aureus* is a very versatile pathogen due to some of its peculiar characteristics including the presence of various colonization factors of cellular barriers, immunomodulatory agents and the secretion of exoproducts.
The growth of *S. aureus* is controlled by quorum sensing and the Agr peptide is one of the molecules that signals QS. When Agr is present it leads to an acute infection while its absence is linked to chronic infections characterized by biofilm. Furthermore, Agr is one of the key molecules of competitive interference between different species of Staphylococci.
Coinfections such as that with *S. aureus* and *P. aeruginosa*, a known biofilm producer, usually have a worse clinical outcome. But if we add one or more commensals to the pathogen these infections also reduce their aggressiveness. Commensal bacteria can protect skin cells from pathogen attacks through competitive interference with *S. aureus* Agr. Coagulase-negative staphylococci (CoNS) produce a wide range of AIPs (auto-inducing peptides) that block quorum sensing.

By studying synthetic models of chronic wounds (SCW), it was discovered that

- different strains of *P. aeruginosa* are distributed differently
- the chronicity-defective strains die predominantly in the center of the microcolonies
- their death is exacerbated by the presence of *S. aureus*.

The cocultures of the two bacteria can therefore have different effects, of coexistence, synergy or competition depending on the defective chronicity. Matched pairs coexist more easily than unmatched ones.
Furthermore, the presence of multiple species within the biofilm alters the bactericidal activity of antimicrobials and *S. aureus* within multispecies biofilms, containing for example commensals such as *Micrococcus luteus* or *S. epidermidis* but also pathogens such as *P. aeruginosa*, are sensitive to much lower concentrations of antibiotic.

Another very interesting data shown by KIM HARDIE in her talk concerns the effect of biocides on antibiotic tolerance within the biofilm. Triclosan, for example, a disinfectant widely used in household and healthcare products, greatly reduces the susceptibility of *S. aureus* to ciprofloxacin and vancomycin.
Triclosan is an inhibitor of fatty acid synthesis and accumulates in the environment and in the human body and traces have been found in urine, blood and nasal secretions. No one has yet verified what effect it may have on models of polymicrobial infections.

KIM HARDIE finally also showed how mutual interference between bacteria can be important in AD. In fact, in *in vivo* studies on animals it has been demonstrated that the presence of Agr produced by *S. aureus* correlates with an inflammation similar to AD and that coagulase negative staphylococci (CoNS) produce a wide range of AIPs (auto-inducing peptides) which block quorum sensing. In particular, AIP-1 and AIP-2 produced by *S. hominis* have been shown to inhibit group 1 Agr of *S. aureus*.

C. Acnes: what did they say about me?

The role of the different strains of *C. acnes* and their relationship with staphylococci in maintaining healthy skin or, when altered, in the pathogenesis of acne is increasingly intriguing

BENJAMIN AL (Global Innovation Manager Life Sciences of Beiersdorf AG), in his report "Skin Microbiome in Health and Disease" spoke about the skin characteristics that influence the growth of *S. epidermidis* and *C. acnes*, with particular reference to hydration and sebum content . For example, *C. acnes* is found primarily in areas of oily skin such as the face and back, while staphylococci are more present in moist areas such as the folds of the limbs. He then underlined that the influences exerted are specific depending on the phylotype (strain) and not common to the same species.

Always **BENJAMIN AL,** for example, showed that co-culture experiments with hundreds of different staphylococcal strains allowed to identify 30 strains that exhibited anti-*C. acnes* activity. Notably, there are staphylococcal strains that selectively exclude acne-associated *C. acnes* strains (e.g., IA1) while coexisting with phylotypes associated with healthy skin (e.g., K, phylotype II).
This regulation would occur through the modulation of antimicrobial activity. Then there are strains of staphylococcus, such as *S. capitis* which appear to inhibit the K strain (phylotype II) of *C. acnes*, which could favor the prevalence of acne-promoting strains. Similarly, some specific strains of *C. acnes* are able to inhibit the antibacterial activity of *S. epidermidis*. *S. epidermidis* can reduce colonization by *S. aureus* through the production of AIPs (auto-inducing peptides) which block quorum sensing and 6-HAP (6-N-hydroxyaminopurine) which inhibits purine synthesis.
A particular strain of *S. epidermidis* HAF242 was shown to be able to inhibit the growth of a strain of *C. acnes* DSM1897 (class A) without interfering with *C. acnes* 30.2.L1 (class D) but at the same time, the different influence of co-culture with these two strains on the regulation of *S. epidermidis* genes (epiA,

psmβ1-3 and agrD) involved in quorum sensing of *S. aureus* was also demonstrated.

HOLGER BRÜGGEMANN (Associate Professor, Department of Biomedicine, Aarhus University, Denmark), in his talk entitled "Skin microbiome interferences and the potential exploitation for acne treatment", spoke about skin dysbiosis, the effect of isotretinoin and the strong impact of Staphylococci in the pathogenesis of the disease.

In a study on 35 acne patients, who had not undergone antibiotic treatments in the last 6 months, subjected to a skin swab they measured the quantity and relative abundance of the populations of *C. acnes* and Staphylococci, highlighting a slight reduction of the former and a significant relative increase of the seconds.
With the SLTS (single-locus sequence typing) technique it is possible to distinguish 10 classes of *C. acnes* which correspond to the phylotypes, classes A-E to the IA1 phylotype, F to the IA2 phylotype, H to the IB phylotype, G to the IC phylotype, K to phylotype II (*C. acnes susp. defendans*) and L to phylotype III (*C. acnes subsp. elongatum*). Therefore, reanalyzing the data at phylotype level, it emerged that at the *C. acnes* level there was a significant reduction in classes H, K and L (phylotypes II and III) and a relative but not significant increase in classes A, C, E and F (phylotype I).
The reduction of *C. acnes* is more evident on the cheek while the reduced alpha-diversity of *C. acnes* is greater on the skin of the back. Analyzing the seven main species of Staphylococci, however, there is a significant reduction in *S. hominis* and *S. warneri* compared to a relative but not significant increase in *S. epidermidis* and *S. aureus*. Staphylococci in general are not reduced in number while for them too the reduction in alpha-diversity is greater at the level of the skin of the back.
What changes significantly is the ratio between *C. acnes* and Staphylococci which goes from 34:1 in healthy subjects to 11:1 in subjects with acne.

Isotretinoin modulates this dysbiosis by causing a slight reduction in Staphylococci but dramatically increasing their diversity through a significant relative reduction in *S. epidermidis* and a significant relative increase in *S. aureus*, but at the same time drastically depletes *C. acnes* by further reducing the ratio *C. acnes* / Staphylococci from 11:1 to 1:1.
Isotretinoin therefore modulates the microbiota by reducing *C. acnes* but also

damages *S. epidermidis* and promotes the growth of *S. aureus* which could be dangerous. Post-isotretinoin strategies must therefore be considered to restore the skin microbiome.
The possibility of selecting and applying beneficial strains of *S. epidermidis* to the skin, in particular those capable of reducing colonization by *S. aureus*, was therefore examined. Through genomic analysis, *S. epidermidis* strains associated with those not associated with pathology were distinguished. Subsequently, those isolated from healthy skin were tested for their antimicrobial activity against *C. acnes* and *S. aureus*, demonstrating that approximately 5% of the strains possess specific activity. Some of these then have a selective inhibition activity on some strains (pathogenic) of *C. acnes* without interfering with the others (beneficial).
Finally, the bacteriocins produced by these strains were studied, discovering that only three of these produce already known bacteriocins while all produce still unknown bacteriocins. A strain of *S. epidermidis* HAF242 was therefore isolated capable of inhibiting the growth of a strain of *C. acnes* DSM1897 (class A) without interfering with *C. acnes* 30.2.L1 (class D) and the different influence of co-culture with these two strains on the regulation of *S. epidermidis* genes involved in quorum sensing was also demonstrated.
The hypothesis is therefore that class D *C. acnes* are able to inhibit the AgrQs system of *S. epidermidis* and its anti-*C. acnes* activity, reaching a situation of "tolerance" that class A does not achieve.
The last step of the study demonstrated that after the topical application of specific strains of *S. epidermidis* (SE1) with anti-*S. aureus* and anti-class A *C. acnes* activity, persistence (but only of the DNA, which does not demonstrate viability) after 7 and 30 days was still high and the *S. aureus* load decreased. However, a poor impact on *C. acnes* was demonstrated, perhaps due to the different skin niche in which the two bacteria proliferate more.

DAN BROWNELL (Senior Director of Research and Development, AOBiome Therapeutics) in the report entitled “B244 Utilizes Multiple Mechanisms of Action to Treat Atopic Dermatitis, a Multifactorial Disease” focused on the characteristics of this single strain of ammonia-oxidizing bacteria spoke not only of AD but also of acne, illustrating *in vitro* and *in vivo* studies which have demonstrated that AOBs are very effective in reducing the proliferation of *C. acnes*, probably also through a strong reduction in sebum production, and phase 2b clinical studies which have demonstrated a significant reduction in the IGA score and inflammatory lesion count at 12 weeks.

This significance remains high not only compared to pre-treatment or placebo but also in trials comparing it to some reference products (such as adapalene) compared to which AOBs always show better tolerability and the absence of side effects. B244 could therefore respond to some unmeet needs of current therapies which often cause dryness or burning, which cannot be used for long periods or which also have systemic side effects.

A **SynFlora poster** showed data on engineering *C. acnes* to produce and secrete in vivo a therapeutic molecule, Neutrophil Gelatinase-Associated Lipocalin (NGAL), capable of modulating sebum production.

Malassezia: what did they say about me?

Unfortunately the fungal component was the great absentee of this congress. Only one Korean poster presented data on the importance of the ratio between *M. restricta* and *M. globosa*, reporting that this, rather than the absolute quantity of the fungus, would correlate with the intensity of the itch.

https://www.sequential.bio/post/malassezia-commensal-pathogen-or-protector

Ammonia-oxidizing bacteria (AOB): what did they say about us?

DAN BROWNELL (Senior Director of Research and Development, AOBiome

Therapeutics) gave a talk titled "B244 Utilizes Multiple Mechanisms of Action to Treat Atopic Dermatitis, a Multifactorial Disease" focused on the characteristics of this single strain of ammonia-oxidizing bacteria. AOBs (ammonia oxidizing bacteria) are already used in sprays for topical use with a wide area of therapeutic applications, with a high safety profile characterized by over 1000 patients treated with 0 side effects and Phase 3 studies on AD and phase 2 on acne and rosacea. Through the oxidation of ammonia, produced 70% in the liver and 30% in the skin, B244 generates various metabolites, with an immunomodulatory and beneficial action for the skin, including in particular nitric oxide, with vasodilatory and anti-inflammatory activities and nitrites with anti-infectious activity. Among the metabolites produced there are some that can be used selectively in different situations including acne, rosacea, AD, inflammation, wrinkles, skin barrier damage, wound healing as well as for their antioxidant action. (see figure)

AOB Secrete Beneficial Skin Metabolites

	Acne	Antioxidant	Rosacea	Atopic Dermatitis	Inflammation	Wrinkles	Skin Barrier	Wound Healing
Met1	✓					✓		
Met2	✓	✓	✓	✓			✓	
Met3	✓		✓	✓	✓	✓	✓	
Met4		✓			✓			✓
Met5								✓
Met6							✓	✓
Met7		✓			✓			

B244 was the subject of a Phase 2b trial in adults with mild to moderate AD and moderate to severe pruritus. The spray was well tolerated and demonstrated improved efficacy over vehicle across all primary, secondary and exploratory endpoints and will be further developed as a novel, natural, fast-acting topical spray treatment for AD and associated pruritus. Interesting to note the effect on itching which remained significant even 4 weeks after the end of treatment.

Regarding acne, he showed *in vitro* and *in vivo* studies which have demonstrated that AOBs are very effective in reducing the proliferation of *C. acnes*, probably also through a strong reduction in sebum production, and phase 2b clinical studies which have demonstrated a significant reduction in the IGA score and inflammatory lesion count at 12 weeks. This significance remains high not only compared to pre-treatment or placebo but also in trials comparing it to some reference products (such as adapalene) compared to which AOBs always show better tolerability and the absence of side effects. B244 could therefore respond to some unmeet needs of current therapies which

- often cause dryness or burning
- cannot be used for long periods
- have systemic side effects

He also presented some data on wound healing, showing how in controls without AOB an initial inappropriate immune response was observed which is not part of the healing process but hinders it and therefore hypothesizing a possible use of AOB also in the treatment of wounds.

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